Toxicogenomics :

Understanding the molecular consequences of low–level toxicant exposure

U.S. Army SBCCOM

Edgewood Chemical Biological Center

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Why is Gene Expression Important?

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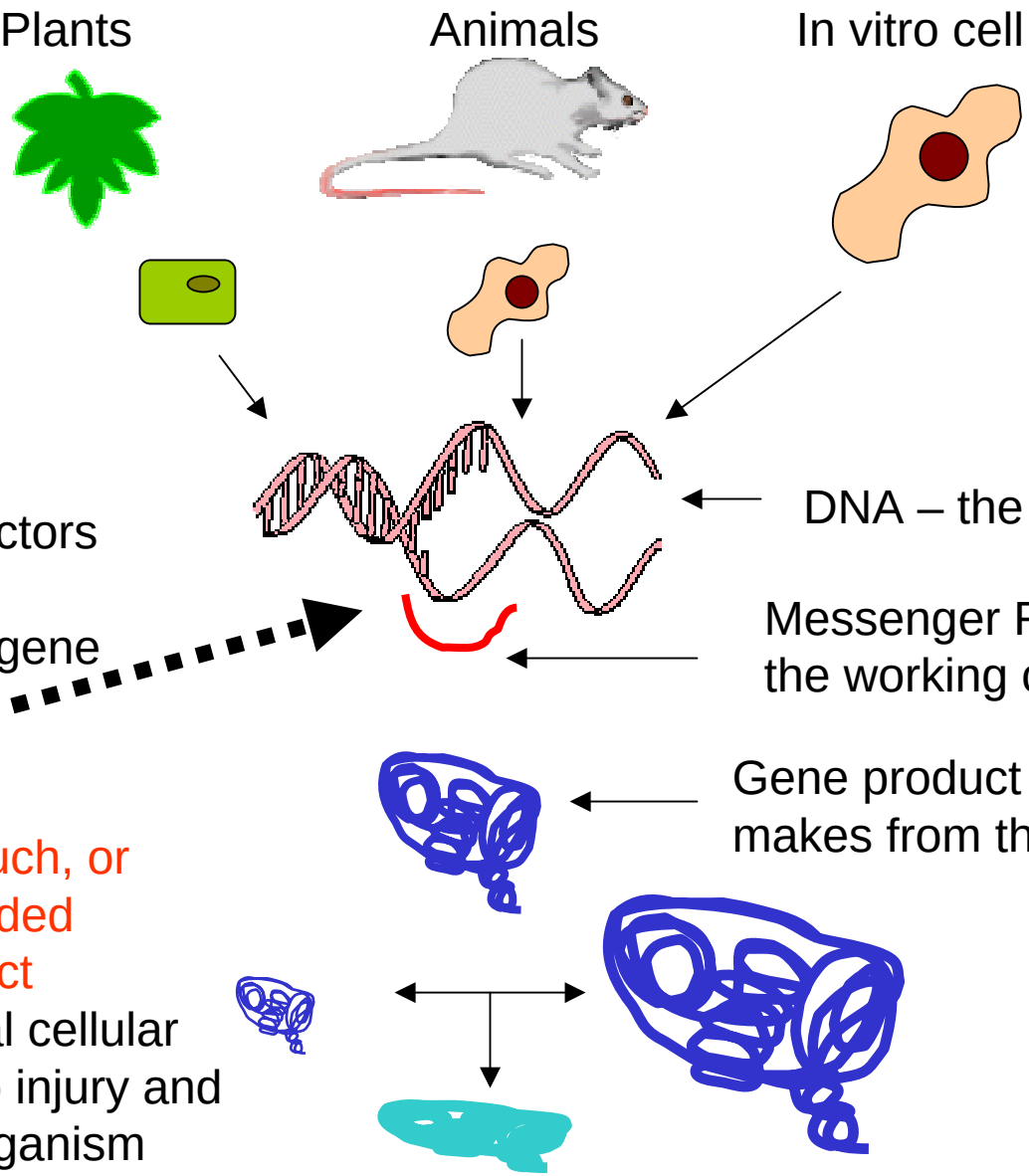

Toxicants,
drugs, and other factors
can influence
the copying of the gene
message

Too little, too much, or
incorrectly coded
gene product
can disrupt normal cellular
processes leading to injury and
disease in the organism

How DNA microarrays (gene chips) work

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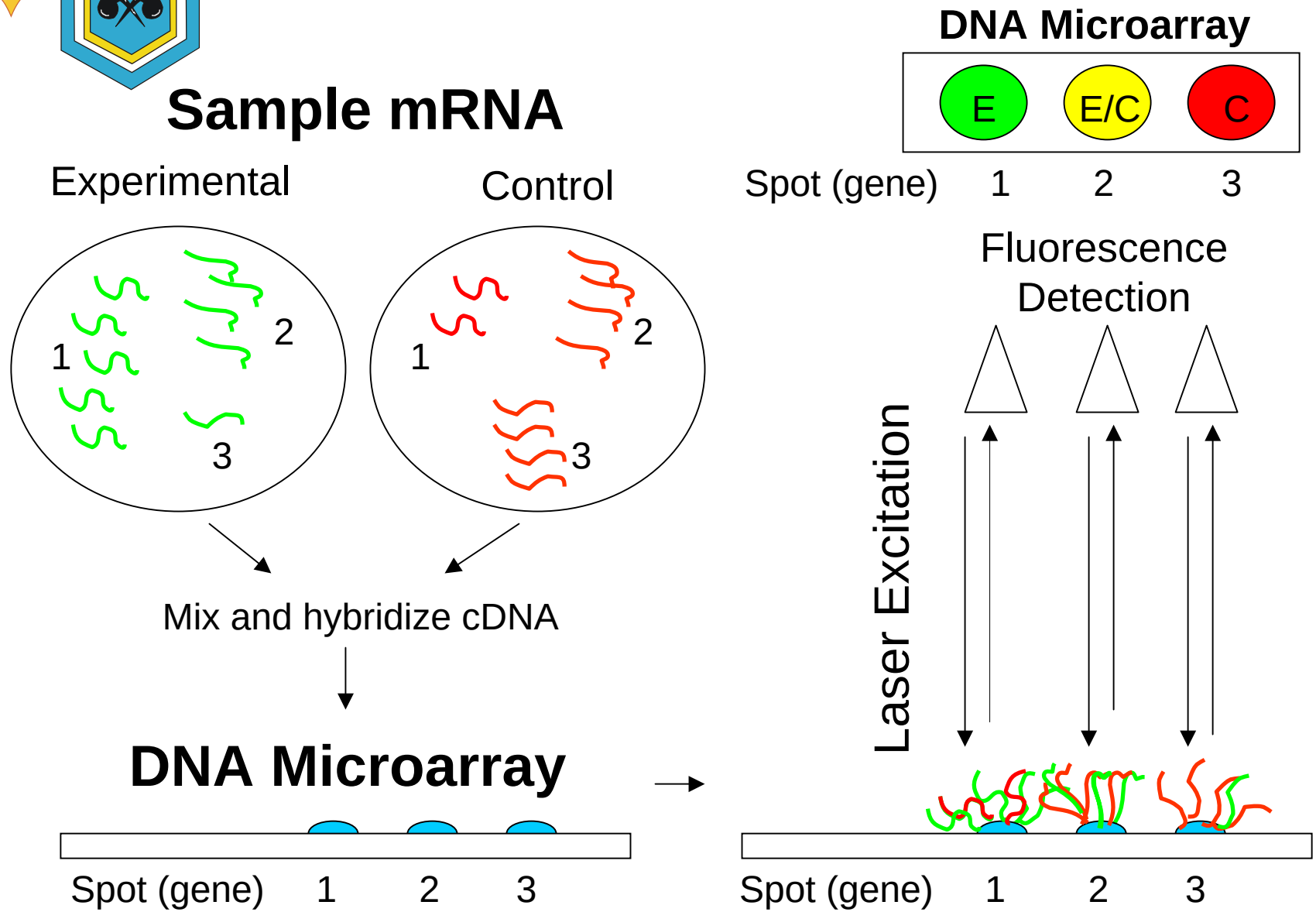

Bioinformatics to analyze the raw data,

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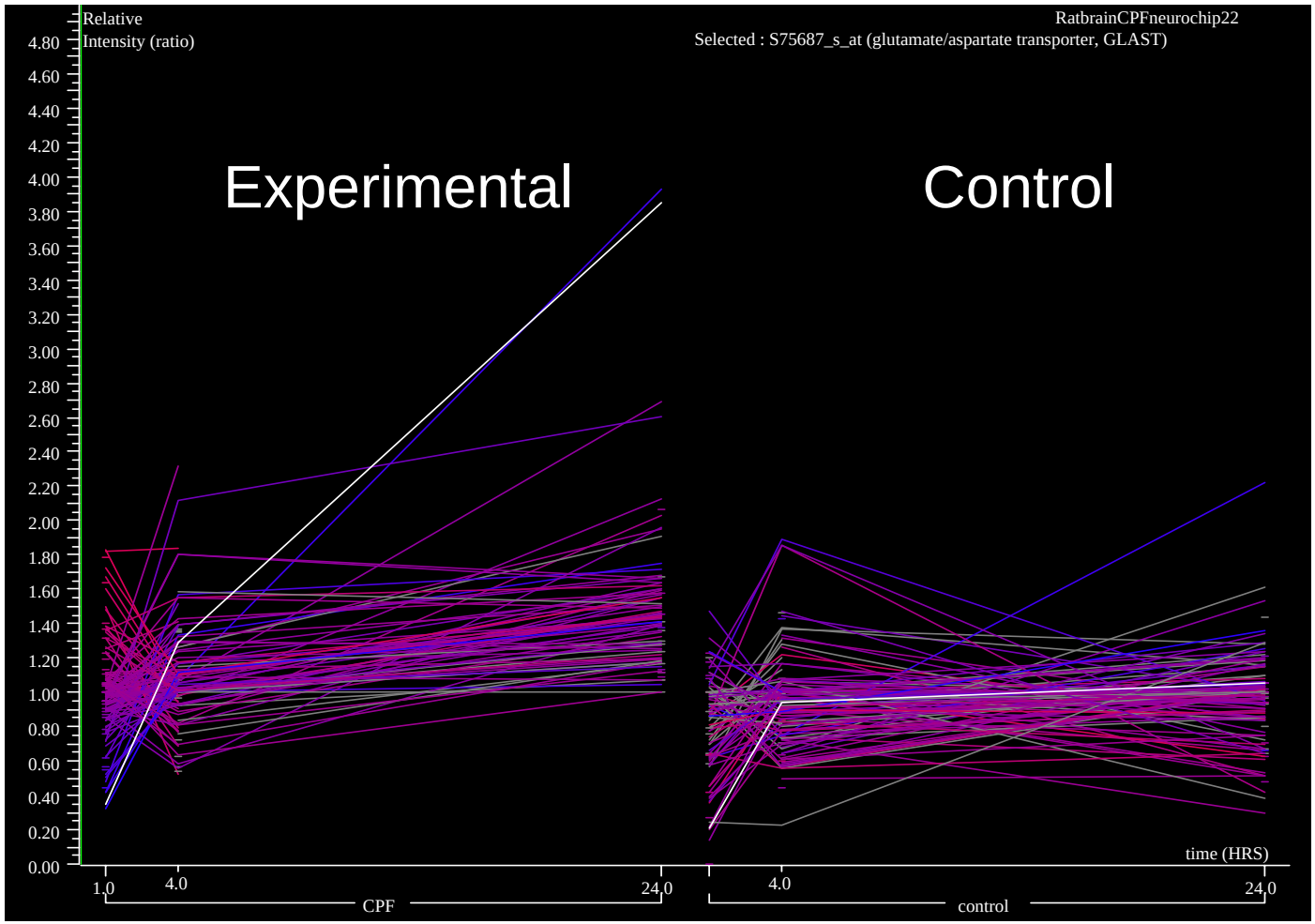
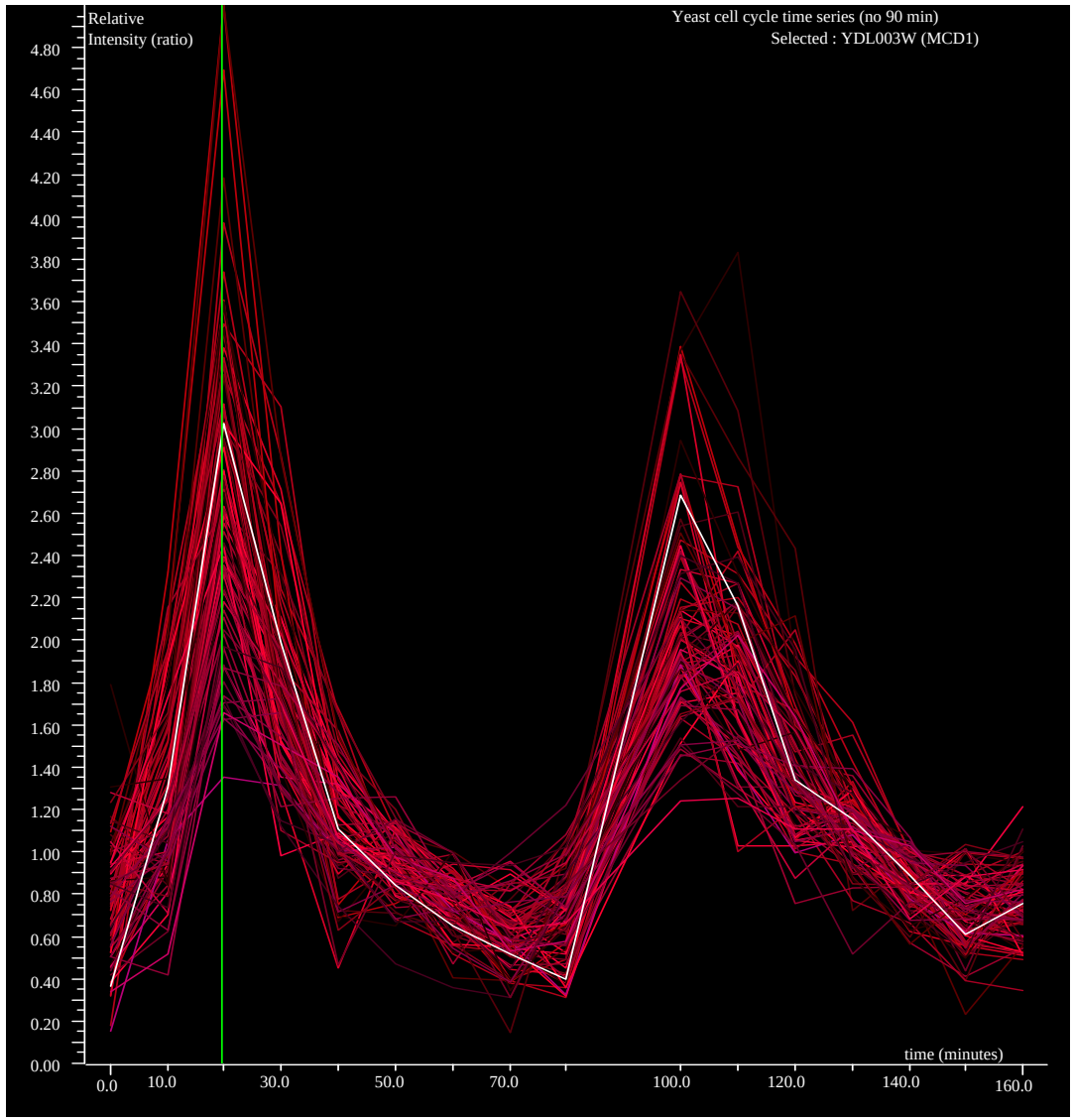


GeneSpring™ software screenshot of gene expression levels measured on an Affymetrix Rat Neurobiology Gene Chip®

… find genes with similar expression profiles,

Expression level



Time

GeneSpring™ software screen shot of gene expression levels measured on an Affymetrix Yeast Gene Chip®

… identify the functional class of a gene,

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GeneSpring™ software screenshot of gene functional classes.

Gene expression was measured on an Affymetrix Yeast Gene Chip®

…and tell us what's known about the gene

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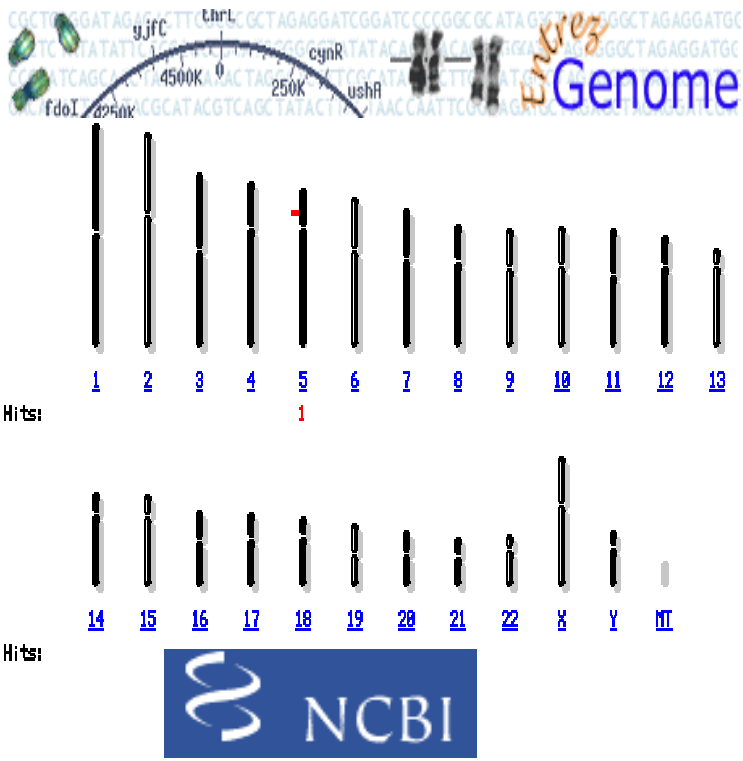
Link to web based databases :

1) Genbank (NCBI)- Gene sequence data
2) Swiss-Prot (ExPASY) – Protein data
3) PubMed (NCBI), ToxNet (NLM)- Literature information



Hear Res 1994 Aug;78(2):235-42

Identification of a glutamate/aspartate transporter in the rat cochlea.

Li HS, Niedzielski AS, Beisel KW, Hiel H, Wenthold RJ, Morley BJ

The neurotransmitter at the synapses between hair cells and spiral ganglion cells in the cochlea is probably L-glutamate or a similar excitatory amino acid. Glutamate uptake by nerve terminals and glial cells is an important component of neurotransmission at glutamatergic synapses of the central nervous system, for providing a reservoir of transmitter or transmitter precursors and the termination of the released glutamate. Hair cell synapses are not surrounded by glial cells, therefore, the uptake mechanism for glutamate in the cochlea may be unique. cDNA was synthesized from total RNA isolated separately from the rat organ of Corti, spiral ganglia, and lateral wall tissues. The expression of a glutamate/aspartate transporter (GLAST) was detected by DNA amplification with the polymerase chain reaction. The other two members of glutamate transporters in this family were not detected by this method….

Scheme of a Toxicogenomics Study

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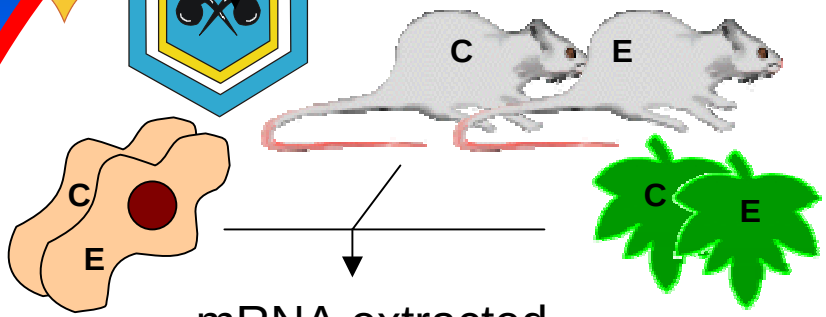
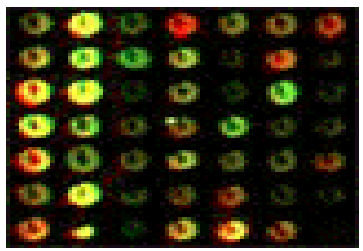


mRNA extracted

fluorescently-labeled cDNA hybridized to gene array



read out of expression levels

array analysis bioinformatics (e.g. GeneSpring™ software & web based databases)

Validation by correlation with other toxicological analyses

- Pathology
- Histology
- Physiology
- Enzymology
- Behavior

Could the gene product play a role in injury or the initiation or promotion of a disease ?

Changes at the gene level can precede disease by years

Confirm results by RT-PCR

Link altered genes to **known functions**

- Metabolism
- Cell cycle regulation
- Neurotransmitter
- Inflammatory response

Applications of Gene Array Analysis

Toxicological and Medical Investigation

- Finding biomarkers for earlier diagnosis of disease
- Finding early warning (“sentinel”) biomarkers for toxicity
- Identifying molecular “signatures” of drug or chemical exposure
- Identifying molecular targets for therapeutic intervention
- Potential to ID genes that predispose individuals to chemical/drug sensitivities
- Advantage in high throughput screening of drugs and chemicals
- Helps in the reduction of animal use

Applications of Gene Array Analysis

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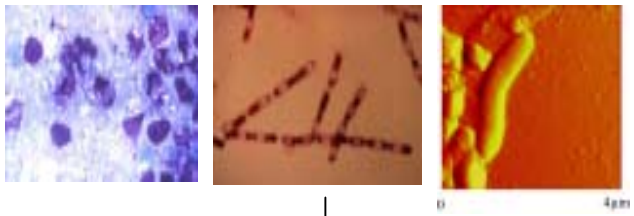
Biological Agent Detection

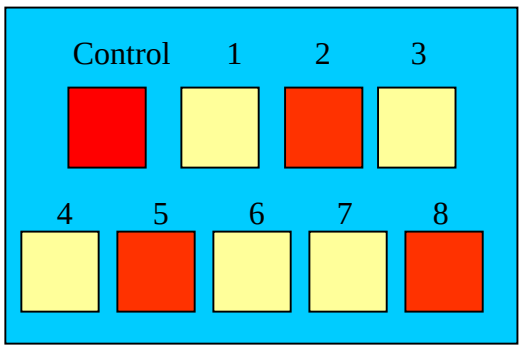
Unknown sample



Extract mRNA from sample,

Convert to fluorescently-labeled cDNA,

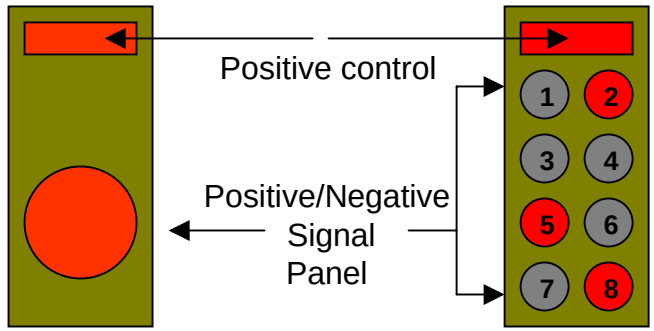
Hybridize cDNA to Bioagent Detection Microarray



Fluorescence Reader

Bioinformatics translates raw data

Detectors



Warfighter Model

Clinician Model

1) B.anthracis, strain X
2) **B. anthracis, strain Y**
3) B. anthracis, strain Z
4) B. abortus
5) **B. melitensis**
6) B. suis
7) E.coli (EPEC)
8) **E.coli (EHEC)**

Our Gene Array Facility can…

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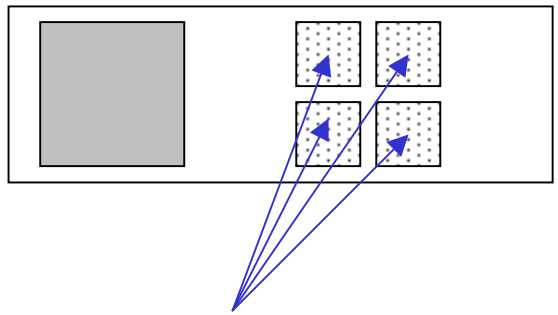
Affymetrix Gene Chip® Custom DNA Microarray

Genes arrayed here



1. Prepare the RNA sample, convert to labeled cDNA, hybridize
2. Carry out all steps necessary to measure the fluorescent signal from the DNA microarray.
3. Determine relative gene expression levels in control and experiment
4. Determine genes with altered expression
5. Analyze altered genes of interest via literature search and laboratory experimentation